

(19)  **Canadian
Intellectual Property
Office**

An Agency of
Industry Canada

**Office de la Propriété,
Intellectuelle
du Canada**

Un organisme
d'Industrie Canada

(11) **CA 2 395 130**

(40) 28.06.2001
 (43) 28.06.2001

(13) **A1**

(12)

(21) 2 395 138

(22) 08.12.2000

(51) Int. Cl. 7: **D06M 16/00, C11D 17/04,
 D06L 3/11, D06M 15/15,
 C11D 3/384, C11D 3/386**

(85) 17.06.2002

(86) PCT/EP00/12530

(87) WO01/046514

(30) 99310427.2 EP 22.12.1999

(71) **UNILEVER PLC,
 Unilever House
 Blackfriars
 EC4P 4BQ, LONDON, XX (GB).**

**SMITH, RICHARD GEORGE (GB).
 PARRY, NEIL JAMES (GB).
 VAN DER LOGT, CORNELIS PAUL ERIK (NL).
 LITTLE, JULIE (GB).
 HOWELL, STEVEN (GB).
 HEMMINGTON, SANDRA (GB).**

(72)

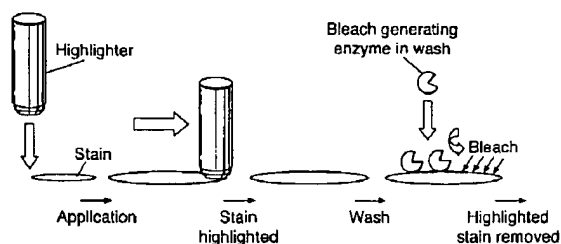
(74) **BERESKIN & PARR**

(54) TRAITEMENT DE TISSUS ET APPAREIL UTILISE A CET EFFET

(54) METHOD OF TREATING FABRICS AND APPARATUS USED THEREIN

(57)

There is provided a method of delivering a benefit agent to a selected area of a fabric for exerting a predetermined activity, wherein the area is pre-treated with a multi-specific binding molecule which has a high binding affinity to said area through one specificity and is capable of binding to said benefit agent through another specificity, followed by contacting said pre-treated area with said benefit agent, to enhance said pre-determined activity to said area. Preferably, the binding molecule is an antibody or fragment thereof, or a fusion protein comprising a cellulose binding domain and a domain having a high binding affinity to another ligand which is directed to said benefit agent. The method is useful for stain removal, perfume delivery, and treating collars and cuffs for wear. Also provided is a device for use in this method, which is a dispenser capable of depositing a multi-specific binding molecule to a selected area of a fabric through a semi-solid wax or soap-stick, spray, aerosol, impregnated brush, gel, or foam, and the like.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
28 June 2001 (28.06.2001)

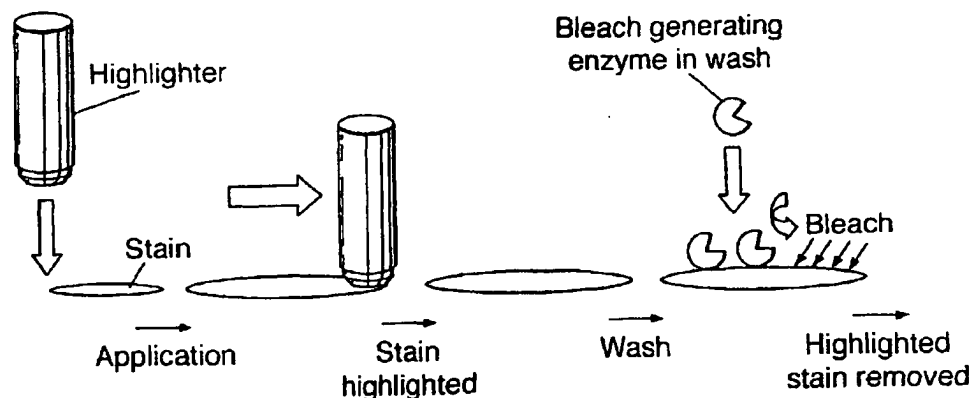
PCT

(10) International Publication Number
WO 01/46514 A1

- (51) International Patent Classification⁷: D06M 16/00, 15/15, C11D 3/384, 3/386, 17/04, D06L 3/11
- (21) International Application Number: PCT/EP00/12530
- (22) International Filing Date: 8 December 2000 (08.12.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
99310427.2 22 December 1999 (22.12.1999) EP
- (71) Applicant (for all designated States except AG, AU, BB, BZ, CA, CY, GB, GD, GH, GM, IE, IL, IN, KE, LK, LS, MN, MW, MZ, NZ, SD, SG, SL, SZ, TT, TZ, UG, ZA, ZW): UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).
- (71) Applicant (for IN only): HINDUSTAN LEVER LTD [IN/IN]; Hindustan Lever House, 165-166 Backbay Reclamation, 400 020 Mumbai (IN).
- (72) Inventors: HEMMINGTON, Sandra; Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB). HOWELL, Steven; Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB). LITTLE, Julie; Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB). VAN DER LOGT, Cornelis, Paul, Erik; Unilever Research Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). PARRY, Neil, James; Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB). SMITH, Richard, George; Unilever Research Port Sunlight, Quarry Road East, Bebington, Wirral, Merseyside CH63 3JW (GB).
- (74) Agent: KAN, Jacob, Hendrik; Unilever Research Vlaardingen, Patent Department, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,

[Continued on next page]

(54) Title: METHOD OF TREATING FABRICS AND APPARATUS USED THEREIN



(57) Abstract: There is provided a method of delivering a benefit agent to a selected area of a fabric for exerting a predetermined activity, wherein the area is pre-treated with a multi-specific binding molecule which has a high binding affinity to said area through one specificity and is capable of binding to said benefit agent through another specificity, followed by contacting said pre-treated area with said benefit agent, to enhance said pre-determined activity to said area. Preferably, the binding molecule is an antibody or fragment thereof, or a fusion protein comprising a cellulose binding domain and a domain having a high binding affinity to another ligand which is directed to said benefit agent. The method is useful for stain removal, perfume delivery, and treating collars and cuffs for wear. Also provided is a device for use in this method, which is a dispenser capable of depositing a multi-specific binding molecule to a selected area of a fabric through a semi-solid wax or soap-stick, spray, aerosol, impregnated brush, gel, or foam, and the like.

WO 01/46514 A1

WO 01/46514 A1



HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- ... With international search report.
— Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHOD OF TREATING FABRICS AND APPARATUS USED THEREINTECHNICAL FIELD

5 The present invention generally relates to the use of multi-specific molecules and in particular multi-specific antibodies for treating fabrics, especially garment, with a benefit agent, and apparatus used therein. More in particular, the invention relates to a method of delivering a benefit agent
10 to a selected area of the fabric for exerting a predetermined activity. In a preferred embodiment, the invention relates to a method of stain bleaching on fabrics which comprises using multi-specific molecules to pretreat the stained area of the fabric.

15

BACKGROUND AND PRIOR ART

Multi-functional, in particular multi-specific agents including bi-specific agents are well known in the art. Gluteraldehyde, for example, is widely used as a coupling or
20 crosslinking agent. The development of bi- and multi-functional antibodies has opened a wide scale of new opportunities in various technological fields, in particular in diagnostics but also in the detergent area.

WO 98/56885 (Unilever) discloses a bleaching enzyme
25 which is capable of generating a bleaching chemical and having a high binding affinity for stains present on fabrics, as well as an enzymatic bleaching composition comprising said bleaching enzyme, and a process for bleaching stains on fabrics. The binding affinity may be formed by a part of the polypeptide
30 chain of the bleaching enzyme, or the enzyme may comprise an enzyme part which is capable of generating a bleach chemical that is coupled to a reagent having the high binding affinity for stains present on fabrics. In the latter case the reagent may be bispecific, comprising one specificity for stain and one
35 for enzyme. Examples of such bispecific reagents mentioned in the disclosure are antibodies, especially those derived from *Camelidae* having only a variable region of the heavy chain

polypeptide (V_{HH}), peptides, peptidomimics, and other organic molecules. The enzyme which is covalently bound to one functional site of the antibody usually is an oxidase, such as glucose oxidase, galactose oxidase and alcohol oxidase, which is capable of forming hydrogen peroxide or another bleaching agent. Thus, if the multi-specific reagent is an antibody, the enzyme forms an enzyme/antibody conjugate which constitutes one ingredient of a detergent composition. During washing, said enzyme/antibody conjugate of the detergent composition is targeted to stains on the clothes by another functional site of the antibody, while the conjugated enzyme catalyzes the formation of a bleaching agent in the proximity of the stain and the stain will be subjected to bleaching.

WO-A-98/00500 (Unilever) discloses detergent compositions wherein a benefit agent is delivered onto fabric by means of peptide or protein deposition aid having a high affinity for fabric. The benefit agent can be a fabric softening agent, perfume, polymeric lubricant, photosensitive agent, latex, resin, dye fixative agent, encapsulated material, antioxidant, insecticide, soil repelling agent, anti-microbial agent, or a soil release agent. The benefit agent is attached or adsorbed to a peptide or protein deposition aid having a high affinity to fabric. Preferably, the deposition aid is a fusion protein containing the cellulose binding domain of a cellulase enzyme. The compositions are said to effectively deposit the benefit agent onto the fabric during the wash cycle.

According to DE-A-196 21 224 (Henkel), the transfer of textile dyes from one garment to another during a washing or rinsing process may be inhibited by adding antibodies against the textile dye to the wash or rinse liquid.

WO-A-98/07820 (P&G) discloses amongst others rinse treatment compositions containing antibodies directed at cellulase and standard softener actives (such as DEQA).

It has now surprisingly been found that a two-step process in which multispecific molecules are bound to pretreat a selected area on a fabric, followed by a step in which a benefit agent is bound to said multispecific molecules will result in a

more efficient targeting of the benefit agent to the selected area of the fabric and, accordingly, to a process in which the benefit agent can exert its aimed activity more efficiently.

Based on this principle, the invention can be practised
5 in various embodiments, which will be explained below.

SUMMARY OF THE INVENTION

According to one aspect of the present invention, there is provided a method of delivering a benefit agent to a selected
10 area of a fabric for exerting a pre-determined activity, which comprises pre-treating said area with a multi-specific binding molecule, said binding molecule having a high binding affinity to said area through one specificity and is capable of binding to said benefit agent through another specificity, followed by
15 contacting said pre-treated area with said benefit agent to exert said predetermined activity to said area.

In another aspect of the present invention a device is provided for use in the method described above for depositing a multi-specific binding molecule to a selected area on a fabric,
20 said binding molecule having a high binding affinity to said area through one specificity and is capable of binding to said benefit agent through another specificity to said agent.

These and other aspects and embodiments will be described in more detail in the description which follows.

25

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a typical treatment of stains according to the present invention by selectively "highlighting" stains with a roll-on pen comprising a composition of a multi-specific
30 binding molecule having a high binding affinity through one specificity to the area of the stain and through another specificity to a bleaching enzyme which is capable of generating a bleaching compound in the wash in the proximity of the stains to be bleached.

35

DETAILED DESCRIPTION OF THE INVENTION

The invention provides in one aspect the deposition of a multi-specific binding molecule to a selected area of a fabric to which it has a high binding affinity through one specificity, in order to enable a benefit agent which is capable of binding to said binding molecule through another specificity to exert a pre-determined activity in close proximity of the targeted area on the fabric.

10 In a first, pre-treating step the binding molecule is directly deposited on the fabric, for example a garment, preferably at relatively high concentration, thus enabling the binding molecule to bind to the fabric in an efficient way. In a second step, the binding molecule is contacted with the benefit agent, which is usually contained in a dispersion or solution, preferably an aqueous solution, thus enabling the benefit agent to bind to the binding molecule through another specificity of said binding molecule.

As used herein, the term "multi-specific binding molecule" means a molecule which at least can associate onto fabric and also capture benefit agent. Similarly, the term "bi-specific binding molecule" as used herein indicates a molecule which can associate onto fabric and capture benefit agent.

25 The multi-specific binding molecule can be any suitable molecule with at least two functionalities, i.e. having a high binding affinity to the fabric to be treated and being able to bind to a benefit agent, thereby not interfering with the predetermined activity of the benefit agent and possible other activities aimed. In a preferred embodiment, said binding molecule is an antibody, or an antibody fragment, or a derivative thereof. If the antibody (or other binding molecule) has very low affinity to the benefit agent and is deposited in large amounts, this may result in non-specific capture of benefit agent, and consequently, benefit agent may be non-specifically deposited onto the fabric resulting in inefficient use of the benefit agent, as is illustrated in Example 2.

The present invention can be advantageously used in, for example, treating stains on fabrics, preferably by bleaching said stains. In a first step, the binding molecule is applied, preferably on the stain. The benefit agent which is then bound
5 to the binding molecule preferably is an enzyme or enzyme part, more preferably an enzyme or enzyme capable of catalyzing the formation of a bleaching agent under conditions of use. The enzyme or enzyme part is usually contacted to the binding molecule (and the stains) by soaking the pre-treated fabric into
10 a dispersion or solution comprising the enzyme or enzyme part. The dispersion or solution which usually but not necessarily is an aqueous dispersion or solution also comprises ingredients generating the bleaching agent, or such ingredients are added later. Preferably, the enzyme or enzyme part and said other
15 ingredients generating a bleach are contained in a washing composition, and the step of binding the enzyme (or part thereof) to the binding molecule and generating the bleaching agent is performed during the wash.

Alternatively, the benefit agent of choice may be added
20 prior to or after washing, for example in the rinse or prior to ironing, depending on its use.

The targeting of the benefit agent according to the invention which in this typical example is a bleaching enzyme, results in a higher concentration of bleaching agent in the
25 proximity of the stains to be treated, before, during or after the wash. Alternatively, less bleaching enzyme is needed as compared to known non-targeting or less efficient targeting methods of treating stains.

Another typical and preferred example of the use of the
30 present invention is to direct a fragrance (such as a perfume), to a selected region of a fabric to deliver or capture the fragrance so that it is released over time. A further typical use of the present invention is treating a fabric where the colour is faded by directing a benefit agent to the area in
35 order to colour that region. Similarly, a damaged area of a fabric can be highlighted to direct a repair of cellulose

fibers. These agents are for example suitably added to the pretreated fabric after washing, in the rinse.

Other applications, such as using fabric softening agents, polymeric lubricants, photoprotective agents, latexes, resins, dye fixative agents, encapsulated materials antioxidants, insecticides, soil repelling agents or soil release agents, as well as other agents of choice, and ways and time of adding the agents to the pre-treated fabric are fully within the ordinary skill of a person skilled in the art.

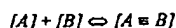
In another embodiment of the invention the benefit agent is preferably applied to said area of a fabric by a dispenser such as a roll-on pen or an impregnated brush, or through a semi-solid wax or soap stick, spray, aerosol, gel (semi liquid), and the like. The deposition can be performed in various ways, for example using a roller, sprayer, stick, brush, aerosol, gel, foam, and the like.

In order to be more fully understood, certain elements of the present invention will be described hereinafter in more detail. Reference is also made to WO-A-98/56885, referred to above, the content of which is incorporated herewith by reference.

1.0 Binding molecules

In the first step according to the invention a multispecific binding molecule is delivered to a predetermined area of a fabric, said binding molecule having a high affinity to said area through one specificity.

The degree of binding of a compound A to another molecule B can be generally expressed by the chemical equilibrium constant K_d resulting from the following reaction:



The chemical equilibrium constant K_d is then given by:

$$K_d = \frac{[A][B]}{[A \equiv B]}$$

Whether the binding of a molecule to the fabric is specific or not can be judged from the difference between the binding (K_d value) of the molecule to one type of fabric, versus
5 the binding to another type of fabric material. For applications in laundry, said material will be a fabric such as cotton, polyester, cotton/polyester, or wool. However, it will usually be more convenient to measure K_d values and differences in K_d values on other materials such as a polystyrene microtitre plate
10 or a specialised surface in an analytical biosensor. The difference between the two binding constants should be minimally 10, preferably more than 100, and more preferably, more than 1000. Typically, the molecule should bind to the fabric, or the stained material, with a K_d lower than 10^{-4} M, preferably lower
15 than 10^{-6} M and could be 10^{-10} M or even less. Higher binding affinities (K_d of less than 10^{-5} M) and/or a larger difference between the one type of fabric and another type (or background binding) would increase the deposition of the benefit agent. Also, the weight efficiency of the molecule in the total
20 composition would be increased and smaller amounts of the molecule would be required.

Several classes of binding molecules can be envisaged which deliver the capability of specific binding to fabrics, to which one would like to deliver the benefit agent. In the
25 following we will give a number of examples of such molecules having such capabilities, without pretending to be exhaustive. Reference is also made in this connection to WO-A-98/56885 (Unilever), the disclosure of which is incorporated herein by reference.

30 The concentration of the binding molecules to be used onto the fabric is not very critical but should generally not be too high, because of cost considerations and non-specificity, as described before. Usually, an upper limit of about 1 mg/ml will suffice. The lower limit will predominantly depend upon the
35 affinity to the highlighted area and will usually be in the range of 1 μ g/ml to 1 ng/ml.

1.1 Antibodies

Antibodies are well known examples of compounds which are capable of binding specifically to compounds against which they were raised. Antibodies can be derived from several
5 sources. From mice, monoclonal antibodies can be obtained which possess very high binding affinities. From such antibodies, Fab, Fv or scFv fragments, can be prepared which have retained their binding properties. Such antibodies or fragments can be produced through recombinant DNA technology by microbial fermentation.
10 Well known production hosts for antibodies and their fragments are yeast, moulds or bacteria.

A class of antibodies of particular interest is formed by the Heavy Chain antibodies as found in Camelidae, like the camel or the llama. The binding domains of these antibodies
15 consist of a single polypeptide fragment, namely the variable region of the heavy chain polypeptide (V_{HH}). In contrast, in the classic antibodies (murine, human, etc.), the binding domain consist of two polypeptide chains (the variable regions of the heavy chain (V_H) and the light chain (V_L)). Procedures to obtain
20 heavy chain immunoglobulins from Camelidae, or (functionalized) fragments thereof, have been described in WO-A-94/04678 (Casterman and Hamers) and WO-A-94/25591 (Unilever and Free University of Brussels).

Alternatively, binding domains can be obtained from the
25 V_H fragments of classical antibodies by a procedure termed "camelization". Hereby the classical V_H fragment is transformed, by substitution of a number of amino acids, into a V_{HH} -like fragment, whereby its binding properties are retained. This procedure has been described by Riechmann et al. in a number of
30 publications (J. Mol. Biol. (1996) 259, 957-969; Protein. Eng. (1996) 9, 531-537, Bio/Technology (1995) 13, 475-479). Also V_{HH} fragments can be produced through recombinant DNA technology in a number of microbial hosts (bacterial, yeast, mould), as described in WO-A-94/29457 (Unilever).

35 Methods for producing fusion proteins that comprise an enzyme and an antibody or that comprise an enzyme and an antibody fragment are already known in the art. One approach is

described by Neuberger and Rabbits (EP-A-0 194 276). A method for producing a fusion protein comprising an enzyme and an antibody fragment that was derived from an antibody originating in *Camelidae* is described in WO-A-94/25591. A method for
5 producing bispecific antibody fragments is described by Holliger et al. (1993) PNAS 90, 6444-6448.

WO-A-99/23221 (Unilever) discloses multivalent and multispecific antigen binding proteins as well as methods for their production, comprising a polypeptide having in series two
10 or more single domain binding units which are preferably variable domains of a heavy chain derived from an immunoglobulin naturally devoid of light chains, in particular those derived from a Camelid immunoglobulin.

An alternative approach to using fusion proteins is to
15 use chemical cross-linking of residues in one protein for covalent attachment to the second protein using conventional coupling chemistries, for example as described in Bioconjugate Techniques, G.T. Hermanson, ed. Academic Press, Inc. San Diego, CA, USA. Amino acid residues incorporating sulphhydryl groups,
20 such as cysteine, may be covalently attached using a bispecific reagent such as succinimidyl-maleimidophenylbutyrate (SMPB), for example. Alternatively, lysine groups located at the protein surface may be coupled to activated carboxyl groups on the second protein by conventional carbodiimide coupling using 1-
25 ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC) and N-hydroxysuccinimide (NHS).

A particularly attractive feature of antibody binding behavior is their reported ability to bind to a "family" of structurally-related molecules. For example, in Gani et al. (J.
30 Steroid Biochem. Molec. Biol. 48, 277-282) an antibody is described that was raised against progesterone but also binds to the structurally-related steroids, pregnanediolone, pregnanolone and 6-hydroxy-progesterone. Therefore, using the same approach, antibodies could be isolated that bind to a whole "family" of
35 stain chromophores (such as the polyphenols, porphyrins, or caretenoids as described below). A broad action antibody such as

this could be used to treat several different stains when coupled to a bleaching enzyme.

1.2 Fusion proteins comprising a cellulose binding domain (CBD)

5 Another class of suitable and preferred binding molecules for the purpose of the present invention are fusion proteins comprising a cellulose binding domain and a domain having a high binding affinity for another ligand. The cellulose binding domain is part of most cellulase enzymes and can be
10 obtained therefrom. CBDs are also obtainable from xylanase and other hemicellulase degrading enzymes. Preferably, the cellulose binding domain is obtainable from a fungal enzyme origin such as *Humicola*, *Trichoderma*, *Thermonospora*, *Phanerochaete*, and *Aspergillus*, or from a bacterial origin such as *Bacillus*,
15 *Clostridium*, *Streptomyces*, *Cellulomonas* and *Pseudomonas*. Especially preferred is the cellulose binding domain obtainable from *Trichoderma reesei*.

In the fusion protein, the cellulose binding domain is fused to a second domain having a high binding affinity to
20 another ligand. Preferably, the cellulose binding domain is connected to the domain having a high binding affinity to another ligand by means of a linker consisting of 2-15, preferably 2-5 amino acids.

The second domain having a high binding affinity to
25 another ligand may, for example, be an antibody or an antibody fragment. Especially preferred are heavy chain antibodies such as found in *Camelidae*.

The CBD antibody fusion binds to the fabric via the CBD region, thereby allowing the antibody domain to bind to
30 corresponding antigens that comprise or form part of the benefit agent.

1.3 Peptides

Peptides usually have lower binding affinities to the
35 substances of interest than antibodies. Nevertheless, the binding properties of carefully selected or designed peptides can be sufficient to provide the desired selectivity to bind a

benefit agent or to be used in an aimed process, for example an oxidation process.

A peptide which is capable of binding selectively to a substance which one would like to oxidise, can for instance be
5 obtained from a protein which is known to bind to that specific substance. An example of such a peptide would be a binding region extracted from an antibody raised against that substance. Other examples are proline-rich peptides that are known to bind to the polyphenols in wine.

10 Alternatively, peptides which bind to such substances can be obtained by the use of peptide combinatorial libraries. Such a library may contain up to 10^{10} peptides, from which the peptide with the desired binding properties can be isolated. (R.A. Houghten, Trends in Genetics, Vol 9, no 6, 235-239).
15 Several embodiments have been described for this procedure (J. Scott et al., Science (1990) 249, 386-390; Fodor et al., Science (1991) 251, 767-773; K. Lam et al., Nature (1991) 354, 82-84; R.A. Houghten et al., Nature (1991) 354, 84-86).

Suitable peptides can be produced by organic synthesis,
20 using for example the Merrifield procedure (Merrifield (1963) J.Am.Chem.Soc. 85, 2149-2154). Alternatively, the peptides can be produced by recombinant DNA technology in microbial hosts (yeast, moulds, bacteria) (K.N. Faber et al. (1996) Appl. Microbiol. Biotechnol. 45, 72-79).

25

1.4 Peptidomimics

In order to improve the stability and/or binding
properties of a peptide, the molecule can be modified by the
incorporation of non-natural amino acids and/or non-natural
30 chemical linkages between the amino acids. Such molecules are called peptidomimics (H.U. Saragovi et al. (1991) Bio/Technology 10, 773-778; S. Chen et al. (1992) Proc.Natl.Acad. Sci. USA 89, 5872-5876). The production of such compounds is restricted to chemical synthesis.

1.5 Other organic molecules

The list on proteins and peptides described so far are by no means exhaustive. Other proteins, for example those described in WO-A-00/40968, which is incorporated herein by
5 reference, can also be used.

It can be readily envisaged that other molecular structures, which need not be related to proteins, peptides or derivatives thereof, can be found which bind selectively to substances one would like to oxidise with the desired binding
10 properties. For example, certain polymeric RNA molecules which have been shown to bind small synthetic dye molecules (A. Ellington et al. (1990) Nature 346, 818-822). Such binding compounds can be obtained by the combinatorial approach, as described for peptides (L.B. McGown et al. (1995), Analytical
15 Chemistry, 663A-668A).

This approach can also be applied for purely organic compounds which are not polymeric. Combinatorial procedures for synthesis and selection for the desired binding properties have been described for such compounds (Weber et al. (1995) Angew.
20 Chem. Int. Ed. Engl. 34, 2280-2282; G. Lowe (1995), Chemical Society Reviews 24, 309-317; L.A. Thompson et al. (1996) Chem. Rev. 96, 550-600). Once suitable binding compounds have been identified, they can be produced on a larger scale by means of organic synthesis.

25

2. The benefit agent

In general, the benefit agent can be captured by the binding molecule and retain at least a substantial part of its desired activity. The benefit agent is chosen to impart a
30 benefit onto the garment. This benefit can be in the form of a bleaching agent (produced by, for example, bleaching enzymes) that can de-colourise stains, fragrances, colour enhancers, fabric regenerators, softening agents, finishing agents/protective agents, and the like. These will be described
35 in more detail below.

2.1 Bleaching enzymes

Suitable bleaching enzymes which are useful for the purpose of the present invention are capable of generating a bleaching chemical.

5 The bleaching chemical may be hydrogen peroxide which is preferably enzymatically generated. The enzyme for generating the bleaching chemical or enzymatic hydrogen peroxide-generating system is generally selected from the various enzymatic hydrogen peroxide-generating systems which are known in the art. For
10 example, one may use an amine oxidase and an amine, an amino acid oxidase and an amino acid, cholesterol oxidase and cholesterol, uric acid oxidase and uric acid, or a xanthine oxidase with xanthine. Alternatively, a combination of a C₁-C₄ alkanol oxidase and a C₁-C₄ alkanol is used, and especially
15 preferred is the combination of methanol oxidase and ethanol. The methanol oxidase is preferably isolated from a catalase-negative Hansenula polymorpha strain. (see for example EP-A-244 920 of Unilever). The preferred oxidases are glucose oxidase, galactose oxidase and alcohol oxidase.

20 A hydrogen peroxide-generating enzyme could be used in combination with activators which generate peracetic acid. Such activators are well-known in the art. Examples include tetraacetylenediamine (TAED) and sodium nonanoyl-oxybenzenesulphonate (SNOBS). These and other related compounds
25 are described in fuller detail by Grime and Clauss in Chemistry & Industry (15 October 1990) 647-653. Alternatively, a transition metal catalyst could be used in combination with a hydrogen peroxide generating enzyme to increase the bleaching power. Examples of manganese catalysts are described by Hage et
30 al. (1994) Nature 369, 637-639.

Alternatively, the bleaching chemical is hypohalite and the enzyme is then a haloperoxidase. Preferred haloperoxidases are chloroperoxidases and the corresponding bleaching chemical is hypochlorite. Especially preferred chloroperoxidases are
35 vanadium chloroperoxidases, for example from Curvularia inaequalis.

Alternatively, peroxidases or laccases may be used. The bleaching molecule may be derived from an enhancer molecule that has reacted with the enzyme. Examples of laccase/enhancer systems are given in WO-A-95/01426. Examples of
5 peroxidase/enhancer systems are given in WO-A-97/11217.

Suitable examples of bleaches include also photobleaches. Examples of photobleaches are given in EP-A-379 312 (British Petroleum), which discloses a water-insoluble photobleach derived from anionically substituted porphine, and
10 in EP-A-035 470 (Ciba Geigy), which discloses a textile treatment composition comprising a photobleaching component.

2.2 Fragrances

The benefit agent can be a fragrance (perfume), thus
15 through the application of the invention it is able to impart onto the fabric or fragrance that will remain associated with the fabric for a longer period of time than conventional methods. Fragrances can be captured by the binding molecule directly, more preferable is the capture of "packages" or
20 vesicles containing fragrances. The fragrances or perfumes may be encapsulated, e.g. in latex microcapsules

2.3 Colour enhancers

The benefit agent can be an agent used to replenish
25 colour on garments. These can be dye molecules or, more preferable, dye molecules incorporated into "packages" or vesicles enabling larger deposits of colour.

2.4 Fabric regenerating agents

30 The benefit agent can be an agent able to regenerate damaged fabric. For example, enzymes able to synthesize cellulose fibre could be used to build and repair damaged fibres on the garment.

35 2.5 Others

A host of other agents could be envisaged to impart a benefit to fabric. These will be apparent to those skilled in

the art and will depend on the benefit being captured at the fabric surface. Examples of softening agents are clays, cationic surfactants or silicon compounds. Examples of finishing agents/protective agents are polymeric lubricants, soil repelling agents, soil release agents, photo-protective agents (sunscreens), anti-static agents, dye-fixing agents, anti-bacterial agents and anti-fungal agents.

3.1 The fabrics

For laundry detergent applications, several classes of natural or man-made fabrics can be envisaged, in particular cotton. Such macromolecular compounds have the advantage that they can have a more immunogenic nature, i.e. that it is easier to raise antibodies against them. Furthermore, they are more accessible at the surface of the fabric than for instance coloured substances in stains, which generally have a low molecular weight.

An important embodiment of the invention is to use a binding molecule (as described above) that binds to several different types of fabrics. This would have the advantage of enabling a single benefit agent to be deposited to several different types of fabric. The invention will now be further illustrated by the following, non-limiting examples.

25

Example 1

Enhanced Stain Removal from Cotton Fabric Using a Stain Highlighter

1.1 Preparation of a Multivalent Antigen Binding Protein

A multivalent antigen binding protein (bihead) was prepared according to methods known in the art; see, for example, WO-A-99/23221. The specificity of the bihead was screened such that it recognised glucose oxidase (Novo Nordisk) and red wine by coating these antigens onto Nunc immunotubes at 37 °C for 1 week. The tubes were then washed with phosphate buffered saline containing 0.01 % (w/v) sodium azide (PBSA) and

then blocked by the addition of a PBSA solution containing bovine serum albumin (2 % w/v), Marvel™ (1 % w/v) and Tween 20 (0.1 % v/v) for 3 hours. Panning of coated tubes were then performed using techniques known in the art.

5

1.2 Preparation of Highlighter Roll-on Sticks and Stained Cotton

Binding molecules were incorporated into roll-on products as follows:

10 The bihead was made up to 5 mg/ml in PBS and to this hydroxypropyl cellulose was added (0.8 % w/v). The solution was mixed thoroughly using a Silverson L4RT homogeniser until the solution became clear (approximately 60 minutes). This solution was then placed in a plastic Sure™ roll-on applicator and the
15 ball fitting secured. As a control a roll-on applicator was prepared containing PBS with hydroxypropyl cellulose.

Red wine (100 µl of Cote du Rhône, Co-op, UK.) was pipetted onto white cotton fabric and allowed to air dry. The stained fabric was then sealed in a foil bag and stored in the
20 dark for at least 4 days until required.

1.3 Application of Bihead to Stained Cotton

The highlighting device was rolled across the cotton surface and in doing so applied the bihead onto the stained
25 area. In parallel, a roll-on applicator only containing PBS and hydroxypropyl cellulose was also used on red wine stained fabric as a control.

1.4 Removal of Stain

30 Following treatment of the stained fabric each cloth was placed in a Petri dish. To the dish 20 ml of PBS containing 0.75 % CoCo 6.5 EO/LAS detergent mix (at a ratio of 2:1 CoCo:LAS) and 1 mg/ml glucose oxidase (Novo Nordisk) at pH 8.1 was added. The Petri-dish was agitated on a rocker for 30 minutes at room
35 temperature and then the solution was discarded. The cotton fabric was washed twice by the addition of PBS containing 0.75 % CoCo/LAS detergent mix and then transferred into a clean Petri

dish. A solution of glucose (10 mM in PBS containing 0.75 % CoCo/LAS detergent mix) was then added and the Petri-dish was incubated for 50 minutes at 37 °C. During this stage glucose is converted into hydrogen peroxide by any glucose oxidase captured
5 by the bihead on the stained fabric. Some Red wine stained fabric was subjected to a wash in PBS containing 0.75 % CoCo/LAS detergent mix without the addition of glucose oxidase or glucose (Wash). Following the 50 minute incubation the fabric was removed from the solution air dried and the change in colour
10 monitored on a ColourEye instrument.

1.5 Analysis of Stain Removal

In the following Table 1, ΔE shows the change in light intensity at 370-650 nm measured against the stained fabric
15 before any treatment. The $\Delta\Delta E$ shows the difference in stain intensity over the wash treated fabric only.

Table 1

	ΔE	Mean ΔE	$\Delta\Delta E$
Bihead/GOx	12.25	12.23	1.30
Bihead/GOx	12.20		
Gox	11.06	11.37	0.44
Gox	11.67		
Wash	11.20	10.93	0.00
Wash	10.66		

20

These results show that the application of bihead in a highlighter roll-on is able to reduce the amount of red wine stain on a cotton surface by 1.3 ΔE .

Example 2

Highlighting Polyester Fabric with Antibody

This example demonstrates how non-specific binding can be used to locate an antibody onto a fabric surface.

5

2.1 Preparation of hCG-alkaline phosphatase conjugate

Human chorionic gonadotrophin ("hCG") (Sigma Chemical Co.), 1 ml of a 2 mg/ml solution of phosphate buffered saline, PBS) and alkaline phosphatase (Boehringer Mannheim, 1 mg/ml of a 10 10 mg/ml solution in PBS) were stirred at room temperature (10 minutes) in a reacti™ vial to allow the reactants to mix. Fresh monomeric glutaraldehyde (Polysciences, 37.5 µl, 10 % solution in distilled water) was added and stirred at room temperature for three hours. The reaction was then quenched and the product 15 stabilised by adding 25 ml of 5 % ovalbumin made up in 50 mM Tris buffer, pH 7.5 containing 0.1 % sodium azide that had been filtered through a 0.22 µm filter. The conjugated hCG was stored at -20 °C until required.

20 2.2 Highlighting fabric surfaces with antibody

Fourteen 2" x 2" swatches of woven polyester were highlighted with a 5 µl droplet of antibody using a Gilson pipette. Seven of the swatches were highlighted with antibody specific for hCG (MAb 3299), serially diluted from 3100 µg/ml to 25 4 µg/ml in 10 mM sodium acetate buffer pH 5 containing 1 mg/ml bovine serum albumin (BSA) and 0.01 % polyoxyethylene sorbitan monolaurate (Tween 20 from Sigma Chemical Co). The other seven swatches were highlighted in the way using antibody specific for oesterone-3-glucuronide (E3G) (MAb 4155). The swatches were 30 incubated for 15 min at room temperature.

2.3 Identifying Highlighted Fabric

Antibody was revealed by incubating the swatches for 15 min with 700 µl hCG alkaline phosphatase conjugate diluted in 35 the sodium acetate buffer detailed in section 1.2. Following 3 washes in 10 ml sodium acetate buffer, each swatch was incubated

with 700 μ l of alkaline phosphatase substrate solution (1 Sigma BCIP/NBT tablet in 10 ml 1 M diethanol amine containing 1 mM MgCl_2 at pH 8.5). After 3 min a purple residue appeared and the swatches were rinsed in water, dried at room temperature then
5 scanned. It was observed that at 12 and 114 $\mu\text{g/ml}$ antibody concentration the fabric could be specifically highlighted. However, as the concentration of antibody is increased to more than 1 mg/ml specificity is lost.

The present disclosure is to be considered as in all
10 respects illustrative and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency (for example using an antibody which binds non-specifically to a fabric and is capable of binding to a benefit agent), are
15 intended to be embraced therein.

CLAIMS

1. A method of delivering a benefit agent to a selected area of a fabric for exerting a pre-determined activity, which
5 comprises pre-treating said area with a multi-specific binding molecule, said binding molecule having a high binding affinity to said area through one specificity and is capable of binding to said benefit agent through another specificity, followed by contacting said pre-treated area with said benefit agent to
10 exert said pre-determined activity to said area.
2. The method of claim 1, wherein said binding molecule is an antibody, an antibody fragment, or a derivative thereof.
- 15 3. The method of claim 1, wherein said binding molecule is a fusion protein comprising a cellulose binding domain and a domain having a high binding affinity to another ligand.
4. The method of any one of the preceding claims, wherein
20 said area of a fabric comprises one or more stains, said pre-determined activity is bleaching activity, and said benefit agent is capable of generating a bleaching agent [under conditions of use].
- 25 5. The method of any one of the preceding claims, wherein said benefit agent is an enzyme or enzyme part capable of catalyzing the formation of a bleaching agent.
6. The method of claim 5, wherein said enzyme or enzyme
30 part is an oxidase or haloperoxidase or functional part thereof.
7. The method of claim 6, wherein said oxidase is selected from the group consisting of glucose oxidase, galactose oxidase and alcohol oxidase.
- 35 8. The method of claim 6, wherein said haloperoxidase is a chloroperoxidase.

9. The method of claim 8, wherein said chloroperoxidase is a vanadium chloroperoxidase.

5 10. The method of claim 9, wherein said vanadium chloroperoxidase is a *Curvularia inaequalis* chloroperoxidase.

11. The method of any one of the preceding claims, wherein said bleaching agent is hydrogen peroxide or a hypohalite, in
10 particular a hypochlorite.

12. The method of any one of the preceding claims, wherein said enzyme part is a laccase or a peroxidase and said bleaching agent is derived from an enhancer molecule that has reacted with
15 the enzyme.

13. The method of any one of the preceding claims, wherein said enzyme part is bound to said binding molecule having a high binding affinity for porphyrin derived structures, tannins,
20 polyphenols, carotenoids, anthocyanins, and Maillard reaction products.

14. The method of any one of the preceding claims, wherein said enzyme part is bound to said binding molecule having a high
25 binding affinity for porphyrin derived structures, tannins, polyphenols, carotenoids, anthocyanins, and Maillard reaction products when they are adsorbed onto the surface of a fabric.

15. The method of claim 14, wherein the fabric is cotton,
30 polyester, polyester/cotton, or wool.

16. The method of claim 2, wherein said antibody or said antibody fragment or said derivative thereof is all of part of a heavy chain immunoglobulin that was raised in *Camelidae* and has
35 a specificity for stain molecules.

17. The method of claim 2, wherein said antibody or said antibody fragment or said derivative thereof bind to chemical constituents which are present in tea, blackberry and red wine including non-pigmented components of stains, for example
5 pectins.

18. The method of claim 3, wherein said ligand binds to chemical constituents which are present in tea, blackberry and red wine including non-pigmented components of stains, for
10 example pectins.

19. The method of any one of the preceding claims, wherein the binding molecule having a high binding affinity has a chemical equilibrium constant K_d for the substance of less than
15 10^{-4} M, preferably less than 10^{-6} M.

20. The method of claim 19, wherein the chemical equilibrium constant K_d is less than 10^{-7} M.

20 21. The method of any one of the preceding claims, wherein said benefit agent is selected from the group consisting of fragrance agents, perfumes, colour enhancers, fabric softening agents, polymeric lubricants, photoprotective agents, latexes, resins, dye fixative agents, encapsulated materials,
25 antioxidants, insecticides, anti-microbial agents, soil repelling agents, soil release agents, and cellulose fiber repair agents.

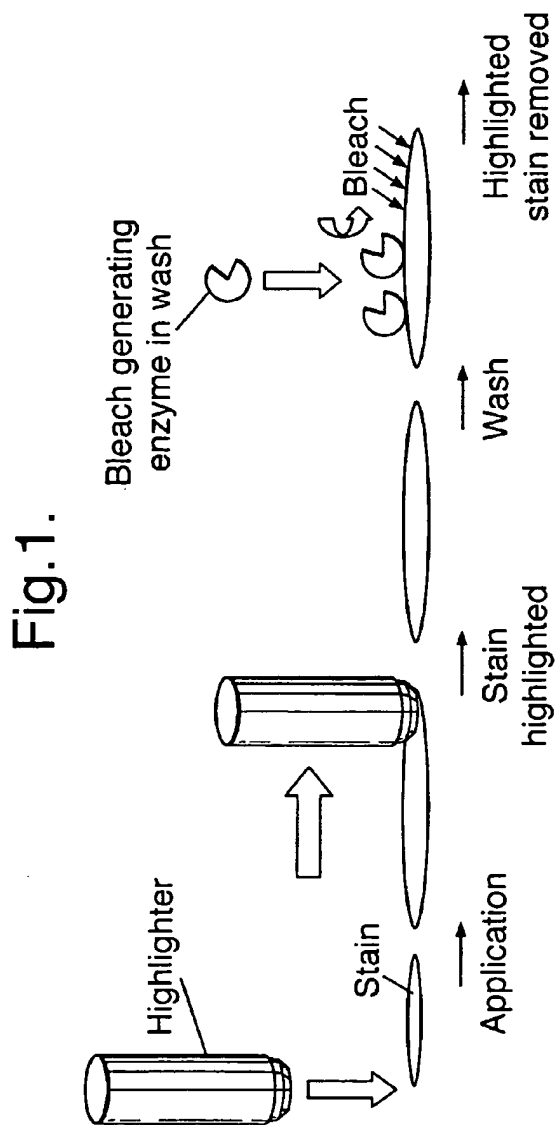
22. The method of any one of the preceding claims, wherein
30 said benefit agent is comprised in an aqueous solution.

23. The method of any one of the preceding claims, wherein said multi-specific binding molecule is applied to said area of a fabric by a roll-on pen, or is incorporated into a semi-solid
35 wax or soap stick, spray, aerosol, impregnated brush, gel, or foam.

24. A device for use in the method of any one of the preceding claims, which is a dispenser capable of depositing a multi-specific binding molecule to a selected area of a fabric, said binding molecule having a high binding affinity through one
5 specificity to said area and is capable of binding to a benefit agent through another specificity, through a semi-solid wax or soap-stick, spray, aerosol, impregnated brush, gel, or foam.

25. The device of claim 24, wherein the dispenser is a roll-
10 on pen or an impregnated brush.

1/1



(19)  **Canadian
Intellectual Property
Office**

An Agency of
Industry Canada

**Office de la Propriété
Intellectuelle
du Canada**

Un organisme
d'Industrie Canada

(11) **CA 2 408 226**

(13) **A1**

(40) 06.12.2001

(43) 06.12.2001

(12)

(21) **2 408 226**

(51) Int. Cl. 7: **C11D 3/384, C07K 19/00,
D06L 1/00, D06M 16/00**

(22) **03.05.2001**

(85) **04.11.2002**

(86) **PCT/EP01/05005**

(87) **WO01/092452**

(30) **00304630.7 EP 31.05.2000**

(71)

**UNILEVER PLC,
Unilever House
Blackfriars
EC4P 4BQ, LONDON, XX (GB).**

(72)

**LITTLE, JULIE (GB).
HEMMINGTON, SANDRA (GB).
PARRY, NEIL JAMES (GB).**

(74)

BERESKIN & PARR

(54) **PROCEDE SERVANT A FIXER UN ANTIGENE A UNE MOLECULE PRESENTANT UNE AFFINITE ELEVEE DE
FIXATION AUDIT ANTIGENE**

(54) **PROCESS FOR BINDING AN ANTIGEN TO A MOLECULE HAVING A HIGH BINDING AFFINITY TO SAID ANTIGEN**

(57)

There is provided a process for binding an antigen to a molecule having a high binding affinity to said antigen, characterised in that the binding is carried out in a medium having a water content of less than 25 % by weight of the total composition.



Office de la Propriété
Intellectuelle
du Canada

Un organisme
d'Industrie Canada

Canadian
Intellectual Property
Office

An agency of
Industry Canada

CA 2408226 A1 2001/12/06

(21) **2 408 226**

(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2001/05/03
(87) Date publication PCT/PCT Publication Date: 2001/12/06
(85) Entrée phase nationale/National Entry: 2002/11/04
(86) N° demande PCT/PCT Application No.: EP 2001/005005
(87) N° publication PCT/PCT Publication No.: 2001/092452
(30) Priorité/Priority: 2000/05/31 (00304630.7) EP

(51) Cl.Int.⁷/Int.Cl.⁷ C11D 3/384, C07K 19/00, D06M 16/00,
D06L 1/00
(71) Demandeur/Applicant:
UNILEVER PLC, GB
(72) Inventeurs/Inventors:
HEMMINGTON, SANDRA, GB;
LITTLE, JULIE, GB;
PARRY, NEIL JAMES, GB
(74) Agent: BERESKIN & PARR

(54) Titre : PROCÉDE SERVANT A FIXER UN ANTIGÈNE A UNE MOLECULE PRESENTANT UNE AFFINITE ELEVEE
DE FIXATION AUDIT ANTIGÈNE

(54) Title: PROCESS FOR BINDING AN ANTIGEN TO A MOLECULE HAVING A HIGH BINDING AFFINITY TO SAID
ANTIGEN

(57) Abrégé/Abstract:

There is provided a process for binding an antigen to a molecule having a high binding affinity to said antigen, characterised in that the binding is carried out in a medium having a water content of less than 25 % by weight of the total composition.

Canada

<http://opic.gc.ca> · Ottawa-Hull K1A 0C9 · <http://cipo.gc.ca>

OPIC · CIPQ 191

OPIC



CIPQ